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Original Research

Evaluation of serum C-terminal telopeptide (CTX) value for the risk of medication related osteonecrosis of the jaws

Clinical evaluation of osteonecrosis with serum CTX

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Abstract
Aim: The aim of this study was to evaluate pre- and post-treatment serum CTX levels of patients treated with bisphosphonate (BP) and the correlation of levels with MRONJ risk.
Material and Methods: Patients treated with BP by the Endocrinology and Oncology Departments were included in this study. Blood samples were taken before and 12 months after BP treatment, and serum CTX values were determined with clinical examinations.
Results: A total of 72 patients were included in the study. Of these patients, 32 had oncological and 40 had endocrinological disorders. The mean CTX value of all the patients was 453.7 (245.7-734.3) pg/ml, in oncology patients, it was 705.9 (398.6-945.6) pg/ml and in endocrine patients, it was 360.4 (209.2-598.2) pg/ml ($p < 0.001$). The mean post-treatment CTX value of the remaining patients was 164.1 (106.9-301.8) pg/ml, and the difference was statistically significant before and after BP treatment ($p < 0.001$). Among the patients included in the study, MRONJ was only observed in one oncology patient 18 months after the initial BP treatment.
Discussion: MRONJ can be prevented by performing all dental treatments before BP treatment and eliminating local risk factors. CTX values should not only be evaluated after BP treatment, but also it may be useful to check before BP treatment to determine the course of the treatment.

Keywords
Bisphosphonate, Osteonecrosis, Jaw, MRONJ, BRONJ, CTX

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Introduction

Bisphosphonates (BP) are antiresorptive agents that are frequently used in metabolic bone diseases, bone metastases of solid tumors, and in the control of skeletal complications of multiple myeloma [1]. BPs are used orally or intravenously and are divided into two main groups with and without amino groups [2]. Nitrogen-containing BPs (pamidronate, alendronate, risedronate, ibandronate, zoledronate) have a higher potential for side effects than BPs without nitrogen (etidronate) [3]. Although both groups show their effects through different enzymes in the intracellular cycle, their activities on bone metabolism are mainly through the inhibition of osteoclast functions [4].

Medication related osteonecrosis of the jaws (MRONJ) is a side effect of BP treatment and characterized by exposed bone localized in the maxilla or mandible for more than 8 weeks in a patient actively or previously using antiresorptive without any history of radiation [5]. MRONJ was first reported in 2003. Since then, it has become a common complication, seen in many published studies [6,7]. Not only BP treatment is responsible for MRONJ, but also denosumab and other antiresorptive agents are found to be associated with MRONJ. Therefore, the term antiresorptive agent-related osteonecrosis of the jaws (ARONJ) also have been used in the literature [8].

Most of the patients using BPs are exposed to intense hospital visits and these patients mostly consist of individuals with low daily vital activity levels. For this reason, methods, which could reduce hospital visits, and be applied easily in all centers, were investigated. Studies in recent years have focused on bone formation and destruction biomarkers in the blood. In addition, the number of genetic research studies has increased. Bone destruction markers may be useful in determining the bone resorption rate in these patients. Cross-linked carboxy terminal telopeptide (CTX) of type 1 collagen is one of the most commonly used markers. Marx et al. firstly, described the CTX as a marker for MRONJ risk in 2008, and a value of less than 100 pg/ml was found to be related to a high risk of MRONJ [9]. A literature survey was performed and, it was concluded that CTX levels of patients under BP treatment were measured before dentoalveolar surgery for the determination of the risk of MRONJ or after the development of MRONJ. However, preoperative CTX levels were unknown in these MRONJ patients. For this reason, the study aimed to evaluate the significance of pre- and post-treatment CTX values in terms of MRONJ risk in patients, who will be started on BP therapy, and to use it as a prognostic marker for early diagnosis.

Material and Methods

This study was carried out with the approval of the Local Ethics Committee of Erciyes University and followed the Declaration of Helsinki on Medical Protocol and Ethics. Erciyes University Scientific Research Projects Unit supported the study (TSA-2015-5965), and carried it out at the Dentistry Faculty, Department of Oral and Maxillofacial Surgery, in cooperation with the Hematology and Endocrinology Departments at the Faculty of Medicine. Patients, who started BP therapy for metabolic bone disorders (endocrinology patients) and to prevent bone metastasis (oncology patients), were included and detailed consent was given by all volunteers. Patients, who did not want to participate voluntarily in the study, refused drug treatment and stated they would not attend the control visits were excluded from the study. A power analysis indicated the adequacy of 72 patients in the study.

Preoperative Procedures

In the first step of the study, to determine the preoperative CTX value of the included patients, approximately 10 ml of blood samples were drawn into the biochemistry tube and the serum was separated by centrifugation at 4000 rpm for 15 minutes in the morning while fasting and the separated serum was stored in a -800. In addition, the clinical and radiological dental examinations of the participants were performed by the same maxillofacial surgeon, and all dental treatments were completed before BP therapy. Patients were referred to the relevant departments to start BP treatment and were recalled for follow-up visits 12 months later.

Postoperative Procedures

Detailed clinical and radiological examinations of the maxillofacial region were performed for possible osteonecrosis and MRONJ risk in the patients six months after BP treatment. Dental treatments were completed in consultation with the relevant departments, if necessary.

Blood samples were taken to determine the postoperative serum CTX levels of the patients 12 months after initial BPs treatment. All patients were informed about MRONJ and were recalled every 6 months for a determination of the earliest possible symptoms of MRONJ and routine dental examination.

ELISA Assay

Preoperative and postoperative blood samples were analyzed with the Enzyme-Linked Immunosorbent Assay (ELISA) with the use of specific kits (Elecsys β-Crosslaps®, Roche Diagnostics, Manheim, Germany) for the determination of preoperative and postoperative serum CTX levels. Enzyme-labeled antibody was inserted into the antibody-coated sample and standard wells. Substrate solution was added to each well and incubated. After incubation, color change occurred in the presence of CTX. The concentration of CTX in the blood samples was determined by comparing the O.D. to the standard curve.

Statistical analysis

Data normality was assessed with the Shapiro-Wilk test and histogram, q-q graphs. The homogeneity of the variance was examined using the Levene test. Independent two samples, the Mann-Whitney U, dependent two samples, and Wilcoxon tests were used for the comparisons. Data were indicated as mean ± standard deviation, median (1^{st}-3^{rd} quartiles), or frequencies (percentages). TURCOSA (Turcosa Analytics Ltd. Co., Turkey, www.turcosa.com.tr) was used for the analysis, and a p-value of less than 5% was considered statistically significant.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

The study included 72 patients. 23.6 % of all patients were men (n = 17) and 76.4% (n = 55) were women. Forty patients in Group 1 (55.5%) were referred by the Endocrinology Department, and 32 patients (44.5%) in Group 2 were referred by the Oncology Department. Osteoporosis is the main reason

for BP treatment in endocrine patients and BP treatment was performed in Group 2 patients to prevent bone metastasis of solid tumors. According to the group of the patients, 90% (n =36) of patients in Group 1 consisted of women, and 47% (n = 15) of the patients in Group 2 were women, and the difference was significant (p<0,001). The mean age of all patients was 61.3 ± 9.8 years (min=34, max= 84). The mean age in Group 1 was 61.4±9.6 and 61.1±10.4 in Group 2 (p=0.83).

When the pre-treatment CTX values of the patients were measured, the mean CTX value of all patients was 453.7 (245.7-734.3) pg /ml. According to the groups, pre-treatment CTX value was 360.4 (209.2-598.2) pg /ml in Group 1 and 705.9 (398.6-945.6) pg /ml in Group 2, with significant difference (p <0.001).

In the second step of the study, 38 patients were excluded from the study. In the endocrinology group, 19 patients were excluded due to discontinuation of the drug treatment (n=9), not coming to the follow-ups (n=5), and withdrawal from the study (n=5). In the oncology group, 19 were excluded because of a short survey of the diseases leading to death in stage 4 cancers. Post-treatment CTX values of the remaining 34 patients were measured after 12 months of initial BP treatment. Twenty-one (62%) patients were in the endocrine group and 13 (38%) were oncology patients. The post-treatment serum CTX value of all patients was 164.1 (106.9-301.8) pg /ml. According to the groups, the post-treatment mean CTX was 231±131.9 pg/ml in Group 1 and 140.1 (100-187.6) pg /ml in Group 2, with no difference (p=0.19).

The mean pre-treatment CTX value of the 34 patients, whose second blood samples were obtained, was 413.3 (234.2-641.6) pg/ ml, and the postoperative CTX value was 164.1 (106.9-301.8) pg/ml, and the difference was significant (p <0.001) (Table 1).

The systemic conditions of the remaining 34 patients were also evaluated. In these patients, 5 patients have used 70

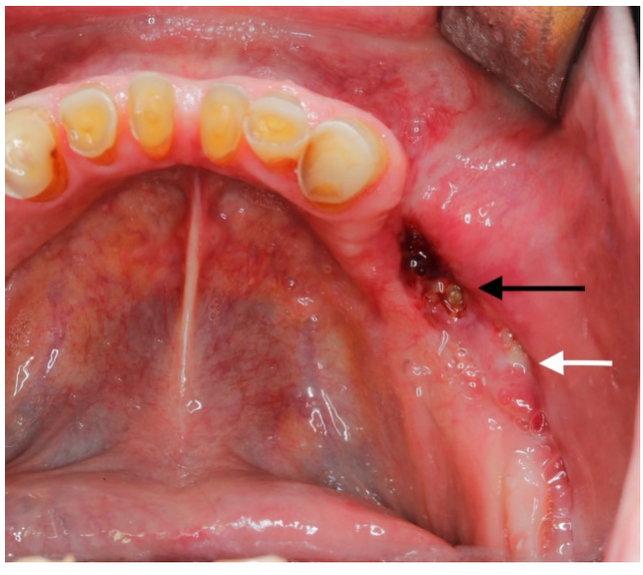

Figure 1. Stage 2 MRONJ on the right mandibular premolar region of the oncology patient. The black arrow indicates the exposed necrotic bone and the white arrow indicates the pus drainage.

Table 1. Mean pre-treatment and post-treatment CTX values of the patients.

	Pre-treatment CTX levels (pg/ml)	Post-treatment CTX levels (pg/ml)	P value
Group 1 (n=21)	428.79±260	231±131.9	<0.001*
Group 2 (n=13)	428.7 (260.8-732.5)	140.1 (100-187.6)	0.012**
Total (n=34)	413.3 (234.2-641.6)	164.1 (106.9-301.8)	<0.001**

*Wilcoxon signed ranks test, **Dependent two sample t-test

Table 2. Data about BP treatment of patients in the second step of the study.

		Endocrinology Patients (n)	Oncology Patients (n)
Bisphosphonates	Alendronate	4	1
	Risedronate	9	0
	Ibandronate	7	5
	Zoledronate	1	7
Route of administration	Orally	20	1 (alendronate)
	Intravenous	1	12
Duration of Therapy	<6 months	1 (intravenous zoledronate)	0
	6-12 months	15	11
	12-24 months	3	2
	24-36 months	2	0
Comorbidities		13	5

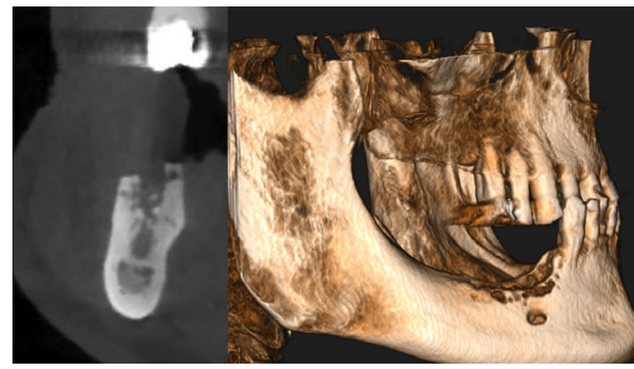

Figure 2. Cone beam computed tomography images of the necrotic bone.

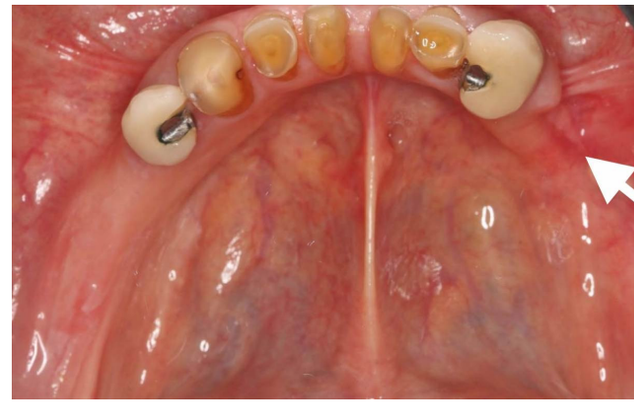

Figure 3. White arrow indicates the complete healing of the necrotic area.

mg alendronate once a week; 9 patients have used 35 mg risedronate once a week, and 7 patients have used 150 mg ibandronate once a month orally. Five patients used 6 mg ibandronate once a month and 8 patients used 4 mg zoledronate once a month intravenously. The mean treatment time was 12 months in Group 1 (min:2- max: 36). All patients in Group 2 had been treated with BPs for 12 months except for 2 patients whose treatment times were 24 months (Table 2).

BP treatment was applied for breast (n=5), colon (n=2), prostate(n=2), lung (n=2), gastric (n=1) and renal cancers (n=1) in Group 2. A total of 18 patients had other systemic conditions such as diabetes mellitus (n=7), hypertension (n=9), thyroid diseases (n=3), and cardiac arrhythmia (n=2). Only one osteoporotic patient had been using steroid treatment (40 mg Fluocortolone once a day for 2 years) in addition to IV BP therapy in Group 1.

MRONJ was observed in only a male patient, who had intravenous BPs treatment (6 mg ibandronate for 12 months) because of a gastrointestinal stromal tumor and colon cancer 18 months after the initial BP treatment. In addition, the patient used 50 mg of sunitinib daily as an antineoplastic agent. Considering the CTX values of the patient, the preoperative CTX value was 428 pg/ml and the postoperative CTX value was 109 pg/ml. Although no dental treatment, such as tooth extraction or periodontal surgery, was performed, MRONJ development was thought to be caused by irritation of the dentures during sunitinib therapy. In the clinical and radiological examination, stage 2 MRONJ was diagnosed according to the AAOMS (American Association of Oral and Maxillofacial Surgeons) criteria and the treatment of MRONJ was performed according to the relevant guideline [5] by the same surgeon (Figures 1, 2). A consultation was performed with the oncology department for a drug holiday and no additional BP treatment was required for the patient who had previously completed BP treatment and sunitinib therapy was continued. After the antimicrobial therapy, the necrotic bone was surgically debrided and complete wound healing was observed 3 months later (Figure 3). The patient was checked for 12 months and no additional MRONJ occurred during sunitinib usage.

Discussion

MRONJ is an uncontrollable consequence in osteoporosis and metastatic cancer patients who are treated with antiresorptive medicines, such as BPs, denosumab, a human IgG2 monoclonal antibody against the receptor activator of nuclear factor-kappa B ligand (RANKL). In cancer patients receiving BP, antiangiogenic drugs, denosumab, and molecularly targeted medicines such as tyrosine kinase inhibitors are also linked to MRONJ or enhance the risk of MRONJ [10]. The incidence of MRONJ is greater in oncological patients because of higher BPs doses than in those with osteoporosis [11]. MRONJ is a difficult and complex clinical condition to treat. Although appropriate treatment protocols are applied according to the clinical staging system, surgical interventions fail due to impairment in wound healing. Therefore, the prevention of MRONJ and early diagnosis are very important in the clinical aspect.

CTX, which is a bone-specific marker, is a breakdown product of type 1 collagen, and serum CTX levels increase in patients with osteoporosis and metastatic bone tumors while it tends to decrease during antiresorptive therapy [12]. Marx et al. have suggested the use of CTX estimation as an indicator of the risk of MRONJ. A value of less than 100 pg/ml was represented as a high risk, 100 to 150 pg /ml, a moderate, and greater than 150 pg/ml, minimal or no risk [9]. However, in different studies, serum CTX values are reported as not a significant predictor of MRONJ risk [13-15].

Determination of serum CTX level is useful in evaluating the effectiveness of osteoporosis treatment and skeletal complications. Studies have shown that high concentrations of serum CTX reduce the survival rate of cancer patients [16]. However, it is also argued that CTX will be higher in cancer patients, due to high collagen destruction and is not a significant determinant [16]. In the present study, the serum CTX concentration in oncology patients was higher than in osteoporotic patients before BP treatment, and the CTX values decreased rapidly in a short time. Therefore, it is recommended to check CTX values in oncology patients at short intervals.

Comorbid conditions in oncology patients are related to a greater risk of MRONJ such as diabetes [5]. Although 7 patients had diabetes mellitus as a comorbid condition in the present study, MRONJ did not develop in any of them. Also, there was one patient in Group 2 who received steroids additionally to IV BP treatment, MRONJ did not occur in this patient.

Furthermore, local factors also have a great impact on MRONJ development as well as systemic conditions. Dentoalveolar surgical procedures, such as tooth extraction, periapical and periodontal surgical procedures, dental implant placement, regional anatomical differences, and accompanying oral problems can be classified as local risk factors [17]. The risk of MRONJ in patients using oral BPs after dental extraction is 0.5% and ranges from 1.6 to 14.8% in IV users [5]. Therefore, such interventions should be avoided in patients using BPs and all dental treatments should be completed before BP treatment. In this study, although, all dental procedures were completed before BP treatment in all patients, MRONJ developed in a patient because of denture irritation. The non-healing mucosal defect caused by dentures is known as a way for oral flora to access the bone. The patient had been using sunitinib daily as an antineoplastic agent after BP treatment and MRONJ occurrence was thought to be associated with sunitinib usage. However, serum CTX values of 15 patients after BP treatment were lower than 150 pg /ml and MRONJ did not develop in any other patients during the follow-up visits for 2 years. Therefore, CTX is not an exact definitive marker, and it may be more favorable to evaluate changes in the pre- and post-treatment CTX levels, rather than only evaluating CTX levels during active BP treatment for MRONJ risk assessment.

There are some limitations of this study. Although the study started with 72 patients, most of the patients left the study for various reasons and the study was completed with 34 volunteers. In addition, although oncology patients were treated with IV BP, all the endocrine patients included in the study were started on oral BP treatment. It was reported that the MRONJ occurrence rate in osteoporotic patients receiving long-term oral BP was reported at 0.1%, which increased to 0.21% when used for more than 4 years [5]. Considering that the possibility

of MRONJ occurrence in patients using oral BP increased after 4 years, it was concluded that the 12-month period determined for this study was insufficient to determine MRONJ risk in endocrine patients. For this reason, clinical studies with more patients, who have the same type of BP treatment, are needed to obtain better results.

In conclusion, CTX values are not only evaluated after BP treatment, but also may be checked before BP treatment to determine the course of the treatment. MRONJ can be prevented by performing all dental treatments before BP treatment and eliminating local risk factors. Thus, clinicians should consult with oral surgeons before BP treatment even if the patient has no dental complaints.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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